


I. Amended Claims

The following claims; as amended in response to the Office Action, are set forth below in clean form. Appended hereto as Exhibit A is a further copy of each of the amended claims which indicates, by bracketing, portions on each that have been deleted and also indicates by underlining, portions of each that are newly added.

43. A method of detecting the presence of the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, in a liquid sample, which method comprises the following steps:

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- a) culturing *Streptococcus pneumoniae* bacteria, to obtain a desired size of culture and harvesting therefrom cells thereof as a wet cell pellet;
 - b) separating from the wet cell pellet the cell wall C-polysaccharide containing not more than 10% protein by performing a series of steps which comprises;
 - (i) suspending the wet cell pellet in an alkaline solution and mixing;
 - (ii) adjusting the pH to an acid pH with a strong acid;
 - (iii) separating the mixture from step (ii) into two layers;
 - (iv) removing the upper layer and adjusting its pH to approximate neutrality;
 - (v) adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

- (vi) adjusting the pH of the product from step (v) to an alkaline pH with a weakly alkaline aqueous solution; and
- (vii) separating out the cell wall C-polysaccharide antigen containing not more than 10% protein;
- c) coupling to a chromatographic column through a spacer molecule the cell wall C-polysaccharide antigen containing not more than 10% protein obtained in step (b);
- d) passing polyvalent antibodies to *Streptococcus pneumoniae* over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and
- e) conducting an immunoassay upon a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its free cell wall C-polysaccharide antigen, by a method which comprises the steps of:
- (i) contacting the sample with the sample-receiving end of a strip of bibulous material, which strip is contained within an ICT device comprising a housing and itself comprises at least two zones, specifically
- (A) a first zone in which has been movably embedded a conjugate of a labelling agent with purified antigen-specific antibodies obtained in step (d) hereof, said labelling agent being selected from among those which manifest a visible color change upon the formation of a

labelled antibody-antigen-fixed antibody reaction product;

and

(B) a second zone having fixedly bound thereto a stripe of unconjugated purified antigen-specific antibodies from step (d) hereof, which zone is equipped with a view window in the ICT device for viewing the appearance of a color characteristic of the massing of the labelling agent upon the formation of the labelled antibody-antigen-fixed antibody reaction product;

- (ii) allowing said liquid sample to flow laterally along said strip of bibulous material to said first zone where it picks up said movably embedded conjugate of a labelling agent with antigen-specific antibodies from step (d) hereof
- (iii) allowing said liquid sample and said conjugate of a labelling agent with antigen-specific antibodies from step (d) hereof to flow laterally together along said strip of bibulous material to said second zone and concomitantly allowing any C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* present in the sample, whether in free or combined form, to react with said conjugate to form labelled antibody-antigen conjugates and

- (iv) within not more than 20 minutes after contacting said sample with said strip of bibulous material, observing through said view window in said ICT device whether a line of color has formed, which line of color is indicative of the massing of said labelling agent along said stripe of unconjugated antigen-specific antibodies from step (d) hereof, which massing takes place as labelled antibody-antigen-fixed antibody reaction products are formed and signifies the presence in the sample of the C-polysaccharide antigen of *Streptococcus pneumoniae*

45. The method of claim 44 wherein the sample is selected from among human urine and human sputum.

50. An immunochromatographic ("ICT") device for the detection of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in a liquid sample, which device comprises a housing containing a strip of bibulous material, which strip of bibulous material has at least
- a). a first zone in which has been movably embedded a conjugate of a labelling agent and antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* and
 - b). a second zone, downstream of said first zone and equipped with a window in the housing, to which second zone is immovably bound a stripe of antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*;

wherein all of said antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* in both zones have been obtained by passing polyvalent antibodies to *Streptococcus pneumoniae* over a chromatographic affinity column to which is coupled a spacer molecule conjugated to a purified cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* obtained from a culture of *Streptococcus pneumoniae* according to a method comprising the steps of:

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- (i) harvesting bacterial cells from the said culture in the form of a wet cell pellet;
 - (ii) suspending the wet cell pellet in an alkaline solution and mixing;
 - (iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;
 - (iv) separating the acidified product from step (iii) into two layers;
 - (v) removing the upper layer and adjusting its pH to approximate neutrality;
 - (vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;
 - (vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution: and
 - (viii) separating out the cell wall C-polysaccharide antigen of

Streptococcus pneumoniae having no more than 10% protein.

Please cancel Claims 52-54.